Hexavalent Vaccines in India: Current Status

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Hexavalent vaccines containing diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, poliomyelitis, and hepatitis B virus antigens have the potential to be used for the primary series in India (6, 10, 14 weeks of age) and the toddler booster dose. Three hexavalent vaccines are available in India: DTwP-Hib/HepB-IPV (wP-hexa), DTaP-IPV-HB-PRP~T(2aP-hexa), and DTaP-HBV-IPV/Hib (3aP-hexa). In the three published phase-3 Indian studies, pertussis 'vaccine response' rates 1 month after a 6-10-14-week primary series were 68.4-75.7% for wP-hexa, 93.8-99.3% for 2aP-hexa, and 97.0-100% for 3aP-hexa; seroprotection rates for the other five antigens were 88.2-100%, 49.6-100%, and 98.6-100%, respectively. Studies outside India show: good immunogenicity/safety after boosting dosing; immune persistence to age 4.5 years (2aP-hexa), 7-9 years (3aP-hexa) (all antigens), and 9-10 and 14-15 years, respectively (hepatitis B); and successful co-administration with other vaccines. Hexavalent vaccines could reduce the number of injections, simplify vaccination schedules, and improve compliance.

Keywords: *Combination vaccines, Acellular Vaccine, Immunization, Pertussis*.

Combination vaccines help to protect against different diseases, offer a solution to the problem of increasing numbers of injections during the first two years of life, and can help simplify vaccination schedules [1,2]. The United States (US) Advisory Committee on Immunization Practices (ACIP) has recommended that combination vaccines are preferred over lower-valent vaccines provided they are licensed and indicated [2]. However, they must not be less immunogenic, less efficacious, or more reactogenic than lower-valent vaccines [1]. Although combination vaccines can be more expensive than their component vaccines, they may offer better economic value if direct and indirect costs of extra injections, delayed or missed vaccinations, and additional handling and storage requirements are considered [2].

A combined vaccine against diphtheria, tetanus, and pertussis (DTP), which contained whole-cell pertussis (DTwP), was introduced in 1948 [1]. Its acellular pertussis equivalent (DTaP) became available in the early 1990s [1]. DTP vaccines have since been combined with other vaccine antigens (*Haemophilus influenzae* type b [Hib], poliomyelitis, hepatitis B [HepB] virus [HBV]) to make pentavalent vaccines such as DPT-HBV-Hib and DTaP-IPV/Hib. Vaccines containing antigens against all six diseases have also been manufactured. The hexavalent vaccines offer the general benefits of higher valent combination vaccines for children, parents and healthcare providers [3-5]. This review discusses the evidence related to the use of the hexavalent vaccines that are currently licensed in India (***Fig.*1**). This narrative review was done following a comprehensive search of electronic databases in English and was undertaken with broad overview of topic-related search in Pub Med, and Embase for the period 2000-2018 with keywords "hexavalent vaccines", "DTP", "immunogenicity", "pertussis", and "India" used alone or in combination. Additional relevant information from prescribing information (and related referred studies within it), government websites, and World Health Organization (WHO) website were also considered.

VACCINATION SCHEDULES IN INDIA

During the first two years of life, the Universal Immunization Program (UIP) in India recommends vaccination against the six diseases covered by the hexavalent vaccines with: oral poliovirus (OPV) and HBV vaccines at birth; pentavalent DPT-HBV-Hib plus OPV at age 6, 10, and 14 weeks; fractional doses (1/5 full dose, intradermal route) of inactivated polio vaccine (IPV) at 6 and 14 weeks; and boosters of OPV and DTP at 16-24 months [6].

For private practitioners, the Indian Academy of Pediatrics (IAP) recommends OPV and HBV vaccines at birth; DTP, HBV, and Hib (or pentavalent vaccine), and intramuscular IPV at age 6-10-14 weeks; and DTP, Hib, and IPV at 16-18 months [7].

Polio Component

The most notable difference between the UIP [6] and IAP [7] schedules is that they recommend fractional and intramuscular IPV, respectively. This relates to recent WHO recommendations on immunization against poliomyelitis [8]. Rarely, the Sabin poliovirus strains in OPV can cause vaccine-associated paralytic polio; they can also mutate to circulating vaccine-derived polio virus (cVDPV), which can cause outbreaks [9]. It was therefore decided to phase out use of OPV and replace them with IPV [10]. As wild type 2 poliovirus has been eradicated worldwide and 90% of circulating vaccine-derived polio virus cases were caused by Sabin type 2 poliovirus [9], the first step in this transfer was to replace trivalent OPV – which contains types 1, 2, and 3 – with bivalent OPV that contains types 1 and 3. However, to provide protection against type 2, at least one full dose of IPV (which contains all three types) also needs to be administered [10,11].

Due to the resultant worldwide requirement for IPV, there are some problems with supply [12]. One way to overcome this issue is to use two fractional intradermal IPV doses, which contain one-fifth of the dose [10], instead of one full intramuscular dose. The worldwide switch to bivalent OPV took place in April 2016 and fractional IPV was introduced into the UIP [6,10,13]. IAP has already taken the next step and switched completely to three full doses of IPV in the primary series, with a booster dose in the second year of life whenever possible [7]. This IPV schedule is consistent with countries that have withdrawn OPV and instead use IPV as a 2- or 3-dose primary series in infancy, with 0 or 1 booster dose at 6-24 months, and 0 or 1 preschool booster dose [11].

Pertussis Component

Vaccines containing whole-cell pertussis (wP) were introduced first [14], and their efficacy varied between 46% and 92% (pooled 78%) [15]. However, wP-containing vaccines were associated with high rates of swelling, induration, fever, and prolonged crying [15]. Due to these reactogenicity issues, many countries switched to vaccines containing acellular pertussis (aP) components [14]. Although aP-containing vaccines had slightly lower pooled efficacy (73%), their efficacy seemed to be more consistent (67-84%) and reactogenicity was lower [15]. While wP-containing vaccines have been used in national programs in several countries including India where they have had an acceptable safety profile, historically aP-containing vaccines have been demonstrated to be less reactogenic than wP-containing vaccines. WHO has reported observed rates of vaccine reactions of DTP vaccines, with wP-containing vaccines associated with 2-6-fold increases in fever ≥38.4°C (15.9% *vs.* 3.7%), redness ≥20mm (16.4% *vs.* 3.3%), swelling ≥20 mm (22.4% *vs.* 4.2%), moderate-to-severe pain (39.9% *vs.* 6.9%), anorexia (35% *vs.* 21.7%), and moderate-to-severe fussiness (41.5% *vs.* 17.1%)%) compared with aP-containing vaccines [16]. wP-containing vaccines are also associated with more serious adverse events than aP-containing vaccines: persistent screaming (3.5% *vs.* 0-0.2%), hyporesponsive hypotonic episodes (57-250 *vs.* 14-62 per 100,000), seizures (6 *vs.* 0.5 per 100,000), and encephalopathy (0.3-5.3 per 1,000,000 *vs.* no documented risk) [16].

aP-containing vaccines can contain 1, 2, 3, or 5 of the following antigens: pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae types 2 and 3 (FIM 2 and 3) [14]. A 2014 review reported that aP-containing vaccines with ≥3 components had higher efficacy against typical whooping cough than those containing 1 or 2 components (84-85% *vs.* 59-78%) [17]. However, other evidence has suggested that efficacy may not simply be related to the number of components [18].

Animal studies using the baboon model suggest that wP-containing vaccines predominantly elicit a Th17/Th1 response which may provide a longer lasting protection than the Th1/Th2 response elicited by aP-containing vaccines; also the predominant Th2 (but lower Th1 and Th17) responses seen with aP-containing vaccines may be less effective in clearing *B. pertussis* and preventing transmission [18]. However, it is important to note that in the baboon studies, the animals were vaccinated with DTaP vaccines without additional antigens such as IPV. This is relevant for the immune response elicited by the hexavalent vaccines because it is described in the literature that the ssRNA of the inactivated polio vaccine has an adjuvant effect *via* TLR7 and TLR8 [19,20]. In a recent mouse model, it was also shown that addition of a TLR7 agonist to an alum-adjuvanted aP vaccine converts it from a Th2-inducing vaccine to a more Th1/Th17-inducing vaccine with higher protective capacity, equivalent to or greater than that of a wP vaccine in a murine model [21]. In view of this, presence or absence of IPV in combination vaccines may have an impact on the immune response and the protective efficacy of the vaccine against pertussis.In some countries that switched from wP- to aP-containing vaccines, there was a resurgence in pertussis several years after the switch [18]. This may have been due to shorter duration of protection and lower impact of transmission seen with aP-containing vaccination. However, pertussis resurgence is not universal and the incidence of pertussis already increased in some countries before the switch to aP vaccines [22,23]. Following evaluation of data from 19

middle/high-income countries, WHO concluded that there was "no evidence of a widespread resurgence" of pertussis [18,24]. Increases in pertussis cases were mostly attributed to naturally occurring cyclic patterns [18,24]. Other factors that could have contributed to the increase in cases included higher pertussis awareness, improved surveillance, and better diagnostic techniques [18,25]. It is noteworthy that no country that switched from wP to aP is considering reverting to wP, probably because this could result in poor acceptance, lower uptake, and increased disease burden even if wP vaccines could potentially offer higher efficacy and longer protection [26]. Further, examination of pertussis incidence trends from 20 countries that switched from wP- to aP-containing vaccines did not indicate a correlation between switch date and pertussis incidence [27].

In 2013, the IAP recommended wP-containing vaccines for the primary series [28], but in the 2018 revision, it stated that either DTwP or DTaP can be used, with the primary aim of increasing vaccination coverage [7]. When vaccinating healthy children in private practice, both benefits and risks must be considered when deciding whether to use aP- or wP-containing vaccines. While both are effective in preventing pertussis, both are associated with waning immunity and require booster doses. Regarding safety, aP-containing vaccines have been associated with a more favorable safety profile than wP-containing vaccines [16,29].

Potential Scheduling of Hexavalent Vaccines in India

Hexavalent vaccines provide the required antigens for the primary series (6-10-14 weeks), but can also be considered for 16-18-month booster vaccination according to the IAP schedule, involving an additional HBV dose [30]. This would likely be acceptable as the US ACIP has recommended that administering extra antigen(s) in a combination vaccine "is often permissible if doing so will reduce the number of injections required" and "an extra dose of Hib or HepB vaccine may be administered as part of a combination vaccine to complete a vaccination series" [2]. Further, five doses of HBV vaccine (birth, three primary, one booster) has been assessed in trials with hexavalent vaccines [31,32], and this number of anti-HBV doses did not appear to affect the vaccine safety profiles. In both trials, there were multi-fold increases in hepatitis B surface antigen (HBs) titers one month after booster vaccination compared to one month after primary vaccinations, regardless of

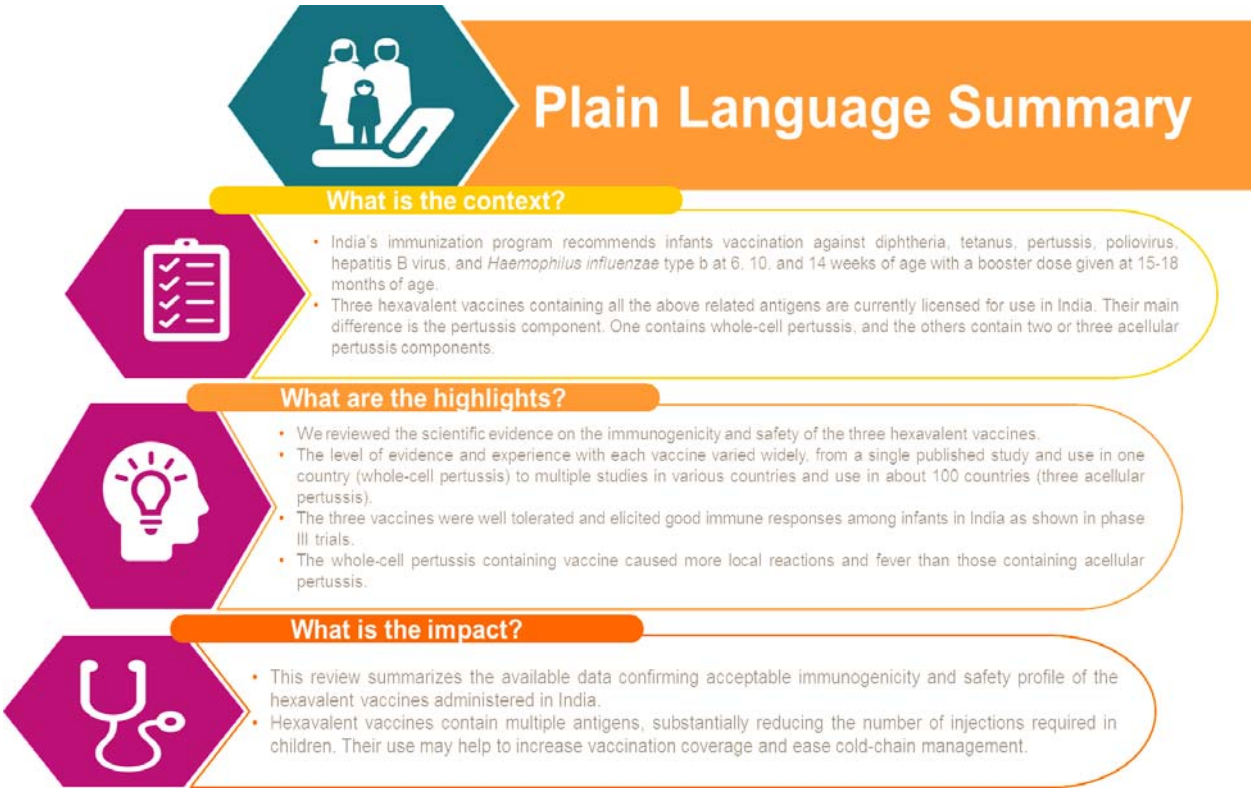

FIG. 1 *The study in context.*

whether a birth dose of HBV vaccine had been administered or not.

HEXAVALENT VACCINES EFFICACY DATA FROM INDIA

Three IPV-containing hexavalent vaccines are available in India: DTwP-Hib/HepB-IPV (Panacea Biotec [33]), DTaP-IPV-HB-PRP~T (Sanofi Pasteur [34]), and DTaP-HBV-IPV/Hib (GSK [35,36]) (***Table* I**).The main difference in their composition is that DTwP-Hib/HepB-IPV contains a wP component [33], DTaP-IPV-HB-PRP~T contains two aP components [34], and DTaP-HBV-IPV/Hib contains three aP components [35,36]. We will therefore refer to them as wP-hexa, 2aP-hexa, and 3aP-hexa, respectively. wP-hexa has been available since 2017 and is only available in India. 2aP-hexa was launched in 2013 and has been available in India since 2016. 3aP-hexa was launched in 2000 and has been available in India since 2018.

Published results are available from one phase 3 wP-hexa study, conducted in India [37], in which it was administered as a primary series at 6-10-14 weeks. 2aP-hexa and 3aP-hexa have been studied in a number of different dosing schedules, including 2- or 3-dose primary series (***Table* I**) and as a booster during the second year of life [34-36] in several countries.

Primary Doses

One phase 3 study in Indian infants for each of the three hexavalent vaccines has been published [37-39] (***Table* II**). In the wP-hexa study, 284 healthy Indian infants were randomized to wP-hexa or pentavalent DTwP-HBV-Hib plus IPV at 6-10-14 weeks [37]; it is unclear whether all infants had received birth doses of HBV and OPV vaccines. In the 2aP-hexa study, 177 healthy Indian infants who had received birth doses of HBV and OPV vaccines received 2aP-hexa at 6-10-14 weeks [38]. In the 3aP-hexa study, 224 Indian infants who had received birth doses of HBV and OPV vaccines were randomized 1:1 to 3aP-hexa at 6-10-14 weeks or 2-4-6 months [39].

Vaccine response (pertussis antigens) and seroprotection (other antigens) results 1 month after primary vaccination with a hexavalent vaccine at 6-10-14 weeks are shown in ***Table* II**. It should be noted that the definitions of vaccine response and seroprotection varied between the studies [37-40]. As there is no established correlate of protection for pertussis, anti-PT or pertussis immunoglobulin G levels were used to assess vaccine response against pertussis components and considered as surrogate markers for protection. Pertussis vaccine response results for wP-hexa were comparable to wP-penta for anti-PT (68.4% *vs.* 66.2%) and pertussis

TABLE I OVERVIEW OF HEXAVALENT VACCINES CURRENTLY AVAILABLE IN INDIA

Vaccine	*DTwP-Hib/HepB-IPV (wP-hexa) [33]*	*DTaP-IPV-HB-PRP~T (2aP-hexa) [34]*	*DTaP-HBV-IPV/Hib (3aP-hexa) [35,36]*
Components			
Diphtheria	DT ≥30 IU	DT ≥20 IU	DT ≥30 IU
Tetanus	TT ≥60 IU	TT ≥40 IU	TT ≥40 IU
Pertussis	Inactivated whole-cell	PT 25 µg	PT 25 µg
	B. pertussis ≥4 IU	FHA 25 µg	FHA 25 µg
			PRN 8 µg
Hepatitis B	HBs 10 µg	HBs 10 µg	HBs 10 µg
Poliovirus	Type 1* 40 DU	Type 1* 40 DU	Type 1* 40 DU
	Type 2# 8 DU	Type 2# 8 DU	Type 2# 8 DU
	Type 3$ 32 DU	Type 3$ 32 DU	Type 3$ 32 DU
Hib	Hib polysaccharide (PRP) 10 µg (TT carrier)	Hib polysaccharide (PRP) 12 µg (TT carrier)	Hib polysaccharide (PRP) 10 µg (TT carrier)
Primary series dosing schedules tested	6-10-14 wk	6-10-14 wk; 2-3-4 or 2-4-6 mo; 3 and 5 mo	6-10-14 wk; 2-3-4 or 2-4-6 or 3-4-5 mo; 2 and 4 or 3 and 5 mo
6-10-14-wk schedule tested	India [37]	India [38], South Africa [42]	India [39], Philippines [31]

**Mahoney strain; #MEF-1 strain; $Saukett strain; DT: diphtheria toxoid; DU: D-antigen unit; FHA: filamentous hemagglutinin; HBs: hepatitis B surface antigen; Hib: Haemophilus influenzae type b; PRN: pertactin; PRP: polyribosylribitol phosphate; PT: pertussis toxoid; TT: tetanus toxoid.*

TABLE II SEROPROTECTION/VACCINE RESPONSE RATES ONE MONTH AFTER PRIMARY VACCINATION WITH THREE DOSES OF HEXAVALENT VACCINE (AT 6-10-14 WEEKS) IN INDIAN INFANTS WHO HAD RECEIVED A BIRTH DOSE OF HBV VACCINE[a]

	wP-hexa [37]		*2aP-hexa [38]*	*3aP-hexa [39,40]*	
	wP-hexa (n=136)	*Control arm (n=136) wP-penta+Polio*	*2aP-hexa (n=156)*	*3aP-hexa (n=105) 6-10-14 wk group*	*Control arm (n=106) 3aP-hexa 2-4-6 mo group*
Seroprotection					
Anti-diphtheria (≥0.01 IU/mL)[b]	NR	NR	99.3 (95.9-100)	NR	NR
Anti-diphtheria (≥0.1 IU/mL)[b]	94.9 (89.7-97.9)	95.6 (90.6-98.4)	49.6 (40.9-58.4)	100 (96.5-100)	100 (96.6-100)
Anti-tetanus (≥0.01 IU/mL)[c]	NR	NR	100 (97.3-100)	NR	NR
Anti-tetanus (≥0.1 IU/mL)[c]	100 (97.3-100)	100 (97.3-100)	NR	100 (96.5-100)	100 (96.6-100)
Anti-HBs (≥10 mIU/mL)	97.8 (93.7-99.5)	97.1 (92.6-99.2)	100 (97.6-100)	100 (96.4-100)	99.0 (94.8-100)
Anti-Polio type 1 (≥1:8)	89.7 (83.3-94.3)	91.9 (86.0-95.9)	100 (97.5-100)	100 (96.3-100)	100 (96.3-100)
Anti-Polio type 2 (≥1:8)	93.4 (87.8-96.9)	94.1 (88.7-97.4)	100 (97.5-100)	100 (95.3-100)	100 (95.9-100)
Anti-Polio type 3 (≥1:8)	88.2 (81.6-93.1)	90.4 (84.2-94.8)	100 (97.5-100)	98.6 (92.7-100)	100 (95.4-100)
Anti-PRP (≥0.15 µg/mL)[d]	100 (97.3-100)	100 (97.3-100)	100 (97.7-100)	99.0 (94.8-100)	99.1 (94.9-100)
Anti-PRP (≥1 µg/mL)[d]	92.7 (86.9-96.4)	89.0 (82.5-93.7)	93.6 (88.5-96.9)	NR	NR
Vaccine response (for pertussis)					
Anti-PT[e,f,g]	68.4 (59.9-76.1)[e]	66.2 (57.6-74.1)	93.8 (88.6-97.1)[f]	100 (96.5-100)[g]	99.0 (94.8-100)
Anti-FHA[f,g]	NR	NR	99.3 (96.3-100)[f]	97.0 (91.6-99.4)[g]	98.0 (93.1-99.8)
Anti-PRN[g]	NR	NR	NR	99.0 (94.8-100)[g]	99.0 (94.8-100)
Pertussis IgG[e]	75.7 (67.6-82.7)[e]	72.8 (64.5-80.1)	NR	NR	NR

*Data are % (95% CI). *Pentavac SD (Serum Institute of India Ltd) and Imovax Polio (Sanofi Pasteur India Pvt. Ltd); [a]It is not clear whether all infants in the wP-hexa study [37] received a birth dose of HBV vaccine; [b]WHO-defined levels for seroprotection against diphtheria are 0.01 IU/mL (some protection) and 0.1 IU/mL (full protection) using a toxin neutralization test [41]. The wP-hexa and 3aP-hexa studies used ELISA [37, 39]; the 2aP-hexa study used a neutralization assay [38]; [c]WHO-defined levels for seroprotection against tetanus are 0.01 IU/mL (neutralization test or modified ELISA) and 0.1-0.2 IU/mL (standard ELISA) [67]. The wP-hexa study used a "specific ELISA" [38]; the 2aP-hexa study "ELISA" [39]; the 3aP-hexa study "standard ELISA" [38]; [d]WHO-defined levels for seroprotection against Hib are 0.15 µg/mL (short-term protection) and 1 µg/mL (long-term protection) [68]; [e]If seronegative pre-vaccination: ≥100 µg/mL for anti-PT or ≥18 IU/mL for pertussis IgG; if seropositive pre-vaccination: ≥4-fold increase in antibody titer level; [f]If pre-vaccination concentrations <4 × LLOQ: ≥4 × LLOQ of the assay (2 IU/mL); if pre-vaccination concentrations ≥4 × LLOQ, ≥pre-vaccination concentration; [g]If seronegative pre-vaccination: ≥5 EL.U/mL; if seropositive pre-vaccination: ≥1-fold increase in antibody concentration; CI: confidence interval; ELISA: enzyme-linked immunosorbent assay; FHA: filamentous hemagglutinin; HBs: hepatitis B surface antigen; HBV: hepatitis B virus; Hib: Haemophilus influenzae type b; IgG: immunoglobulin G; LLOQ: lower limit of quantification; NR: not reported; PRN: pertactin; PRP: polyribosylribitol phosphate; PT: pertussis toxoid.*

immunoglobulin G (75.7% *vs.* 72.8%); seroprotection rates for the other antigens were 88.2-100% [37] (***Table II***). For 2aP-hexa, pertussis vaccine response results were 93.8% (anti-PT) and 99.3% (anti-FHA) [38]. Seroprotection rates were >99% for most antigens. Diphtheria seroprotection rates were 99.3% and 49.6%, respectively based on anti-diphtheria antibodies cut-off of 0.01 IU/mL and the WHO-recommended full protective cut-off (0.1 IU/mL) [38,41]. For 3aP-hexa, vaccine response rates for the three pertussis antigens were 97.0-100% and seroprotection rates for the other five antigens were 98.6-100% [39].

In the study that compared wP-hexa with pentavalent DTwP-HBV-Hib plus IPV, immunogenicity results were comparable with both regimens [37]. Similarly, in the study that compared two different dosing schedules (6-10-14 weeks and 2-4-6 months) of 3aP-hexa, immunogenicity results were similar with both schedules [39].

Safety results for the three Indian studies are summarized in ***Table* III** [37-40]. The most common solicited local adverse events (AEs) were pain/tenderness (wP-hexa and 2aP-hexa) and pain (3aP-hexa); while the most common solicited systemic AEs were fever (wP-hexa), irritability (2aP-hexa), and temperature (3aP-hexa) [37-40]. Serious adverse events were rare (<2% in each study) and none were judged to be related to vaccination [37-39]. All three studies reported that the hexavalent vaccines were well tolerated [37-39].

In the study that compared wP-hexa with pentavalent DTwP-HBV-Hib plus IPV, reactogenicity and safety results were comparable with both regimens [37]. In the study that compared two different dosing schedules of 3aP-hexa, safety results were similar, although pain was more often reported in the 6-10-14-week group *vs*. the 2-4-6-month group (25.2% vs 13.4%) [39].

Booster Dose

Published studies that have tested the immune response to a booster dose of hexavalent vaccine in Indian infants are not available but two studies – one for 2aP-hexa in South Africa [32] and one for 3aP-hexa in the Philippines [31] – have reported on the immune response after the booster dose following a 6-10-14-week primary schedule. In the first part of the South African study, infants were randomized to 2aP-hexa (with [*n*=143] or without [*n*=286] birth HBV) or DTwP-Hib plus HBV plus OPV vaccines (*n*=286) at 6-10-14 weeks [42]. Among infants who received 2aP-hexa, those who received the birth HBV vaccine dose were more likely to obtain anti-HBs ≥10 mIU/mL (99.0% vs 95.7%) [42]. In the second part of the study, infants received the same vaccine(s) as boosters at 15-18 months of age [32]. Seroprotection rates one month after the booster were 100% for all antigens apart from the pertussis antigens, for which vaccine response rates were 93.9% (anti-PT) and 94.7% (anti-FHA) [32] (***Table* IV**).

In the Philippines study, 320 minfants were randomized to 3aP-hexa at 6-10-14 weeks with (*n*=160) or without (*n*=160) birth HBV; they then received the hexavalent vaccine at 12-15 months of age [31]. Infants

TABLE III ADVERSE EVENTS AFTER VACCINATION WITH HEXAVALENT VACCINE AT 6-10-14 WEEKS IN INDIAN INFANTS[a]

	wP-hexa [37]		*2aP-hexa* [38]	*3aP-hexa* [39, 40]	
	wP-hexa (n=142) 3 doses	*Control arm (n=142) wP-penta + Polio*3 doses*	*2aP-hexa (n=177) Post-dose 3*	*3aP-hexa(n=111) 6–10–14 wk group*	*Control arm (n=112)3aP-hexa 2–4–6 mo group*
Solicited local AEs					
Pain/tenderness	50.7	52.1	30.5 (23.7-37.9)	25.2 (17.5-34.4)	13.4 (7.7-21.1)
Grade 3	NR	NR	NR	1.8 (0.2-6.4)	0.9 (0.0-4.9)
Swelling	24.6	15.5	14.9 (10.0-21.1)	7.2 (3.2-13.7)	8.0 (3.7-14.7)
Grade 3	NR	NR	NR	0.9 (0-4.9)	0.9 (0-4.9)
Redness/erythema	19.0	9.2	7.5 (4.0-12.4)	5.4 (2.0-11.4)	1.8 (0.2-6.3)
Grade 3	NR	NR	NR	0 (0-3.3)	0 (0-3.2)
Solicited systemic AEs					
Fever/temperature	57.0	52.1	19.0 (13.4-25.6)	15.3 (9.2-23.4)	15.2 (9.1-23.2)
Grade 3	NR	NR	0 (0-0.2)	0 (0-3.3)	0.9 (0.0-4.9)
Irritability/restlessness/fussiness	7.7	7.7	36.2 (29.1-43.8)	11.7 (6.4-19.2)	8.9 (4.4-15.8)
Grade 3	NR	NR	0.6 (0-3.2)	0 (0-3.3)	0 (0-3.2)
Vomiting	1.4	0.7	14.9 (10.0-21.1)	NR	NR
Grade 3	NR	NR	0 (0-0.2)	NR	NR
Sleepiness/drowsiness	0.7	1.4	13.2 (8.6-19.2)	0 (0-3.3)	1.8 (0.2-6.3)
Grade 3	NR	NR	1.1 (0.1-4.1)	0 (0-3.3)	0 (0-3.2)
Loss of appetite	0	1.4	10.9 (6.7-16.5)	1.8 (0.2-6.4)	4.5 (1.5-10.1)
Grade 3	NR	NR	0 (0-0.2)	0 (0-3.3)	0 (0-3.2)
Acute allergic reaction	0.7	0	NR	NR	NR
Grade 3	NR	NR	NR	NR	NR
Unsolicited AEs	0.7	1.4	20.3	35.7	22.3
Grade 3	NR	NR	NR	0	0
SAE	0.7	0	1.7	1.8	2.7

*Data are % any grade (% grade 3); *Pentavac SD (Serum Institute of India Ltd) and Imovax Polio (Sanofi Pasteur India Pvt. Ltd); [a]During 4 [37], 7 [38], or 4 days [39, 40]; AE: adverse event; CI, confidence interval; m: months; NR: not reported; SAE: serious adverse event.*

who received the birth HBV vaccine dose were significantly more likely to obtain anti-HBs ≥10 mIU/mL after the primary series (98.5% *vs.* 77.7%) and after the booster (99.1% *vs.* 90.0%) than those who did not. Among those who received a birth dose of HBV, vaccine response (pertussis antigens) and seroprotection (other antigens) rates one month after the booster dose were all >99% [31] (***Table* IV**).

Pertussis Efficacy

For most of the antigens included in hexavalent vaccines, generally accepted seroprotective cut-offs are available, and these can be used to imply vaccine efficacy. However, there is no defined correlate of protection for pertussis, so efficacy has to be assessed in clinical studies. No studies have directly assessed the efficacy of any of the three hexavalent vaccines against pertussis due to ethical and feasibility considerations, which is why the current vaccines are licensed by the regulators based on immunological non-inferiority *vs.* historical vaccines or current standard of care. However, in a 3-dose primary series study using a DTaP vaccine with a DTaP component similar to 2aP-hexa's in a highly endemic country (Senegal), vaccine efficacy against WHO-defined typical pertussis (≥21 days of paroxysmal cough) was 74% in DTaP arm and 92% in DTwP arm [43]. Similarly, the efficacy of 3-dose primary immunization with DTaP (*Infanrix*; GSK) with a DTaP component similar to 3aP-hexa's has been reported to be 88.7% against typical pertussis (≥21 days of spasmodic cough with confirmed *Bordetella pertussis*) in a prospective household contact study in Germany [44]; while in an Italian study, 86% efficacy was shown up to 60 months after completion of a 3-dose primary series [45]. As the pertussis immune response to 3aP-hexa is equivalent to that of the DTaP vaccine (*Infanrix*; GSK), the protective efficacy of the two vaccines is expected to be equivalent [35, 36].

Long-term Immune Response

To our knowledge, there are no long-term immune persistence data for wP-hexa or for any of the three vaccines in Indian subjects. For 2aP-hexa, seroprotection rates at age 4.5 years after 3-dose primary series and a booster dose (6-10-14 weeks and 15-18 months or 2-4-6 months and 12-24 months) are: 97.0-100% (anti-

TABLE IV SEROPROTECTION/VACCINE RESPONSE RATES 1 MONTH AFTER BOOSTER VACCINATION IN CHILDREN WHO HAD RECEIVED A BIRTH DOSE OF HBV VACCINE AND THREE DOSES (AT 6-10-14 WEEKS) OF HEXAVALENT VACCINE

Vaccine, Country	*2aP-hexa [32], South Africa*			*3aP-hexa [31], Philippines*	
	15-18			*12-15*	
Age at booster dose (mo)	*Group 1 (n=218) primary series of DTaP-IPV-Hep B-PRP-T, with no HBV at birth*	*Group 2 (n=219) primary series of DTwP-Hib+hepatitis B+OPV, with no HBV at birth*	*Group 3 (n=130) primary series of DTaP-IPV-Hep B-PRP-T, with HBV at birth*	*No HBV at birth (n=111)*	*HBV at birth (n=111)*
Seroprotection[a]					
Anti-diphtheria (≥0.1 IU/mL)	100 (98.1-100)	99.0 (96.4-99.9)	100 (96.7-100)	99.0 (94.6-100)	100 (96.7-100)
Anti-tetanus (≥0.1 IU/mL)	100 (98.2-100)	100 (98.2-100)	100 (96.8-100)	99.0 (94.6-100)	99.1 (95.0-100)
Anti-HBs (≥10 mIU/mL)	98.5 (95.6-99.7)	NA	100 (96.8-100)	90.0 (82.4-95.1)	99.1 (95.0-100)
Anti-Polio type 1 (≥1:8)	100 (98.1-100)	97.4 (94.0-99.1)	100 (96.6-100)	100 (95.8-100)	100 (95.9-100)
Anti-Polio type 2 (≥1:8)	100 (98.1-100)	100 (98.1-100)	100 (96.6-100)	100 (95.7-100)	100 (95.8-100)
Anti-Polio type 3 (≥1:8)	100 (98.1-100)	98.9 (96.2-99.9)	100 (96.6-100)	100 (95.6-100)	100 (95.8-100)
Anti-PRP (≥0.15 µg/mL)	100 (98.2-100)	100 (98.2-100)	100 (96.8-100)	100 (96.4-100)	100 (96.7-100)
Anti-PRP (≥1 µg/mL)	98.5 (95.7-99.7)	98.5 (95.7-99.7)	100 (96.8-100)	99.0 (94.6-100)	99.1 (95.1-100)
Vaccine response (for pertussis)					
Anti-PT[b,c]	94.8 (90.0-97.7)	83.5 (76.0-89.3)	93.9 (87.3-97.7)[b]	99.0 (92.7-99.7)	100 (96.5-100)[c]
Anti-FHA[b,c]	91.2 (85.7-95.1)	96.5 (92.0-98.9)	94.7 (88.0-98.3)[b]	97.9 (94.6-100)	100 (95.0-100)[c]
Anti-PRN[b,c]	NR	NR	NR	99.0 (94.4-100)	99.1 (96.6-100)[c]

Data are % (95% CI) unless otherwise indicated; [a]Please see footnotes to Table II for details about seroprotection cut-offs. The 2aP-hexa study used seroneutralization for diphtheria, ELISA for tetanus [32]; the 3aP-hexa study used standard ELISA for both [31]; [b]≥4-fold increase vs pre-booster; [c]If seronegative pre-booster: appearance of antibodies; if seropositive pre-booster: ≥2-fold increase in antibody concentrations or titers; CI: confidence interval; ELISA: enzyme-linked immunosorbent assay; FHA: filamentous hemagglutinin; HBs: hepatitis B surface antigen; HBV: hepatitis B vaccine; PRN: pertactin; PRP: polyribosylribitol phosphate; PT: pertussis toxoid.

diphtheria ≥0.01 IU/mL), 57.2-75.3% (anti-diphtheria ≥0.1 IU/mL), 100% (anti-tetanus ≥0.01 IU/mL), 80.8-89.5% (anti-tetanus ≥0.1 IU/mL), 73.3% or 92.3-96.1% (anti-HBs ≥10 mIU/mL without or with birth HBV, respectively), 98.8-100% (anti-PRP ≥0.15 µg/mL), 22.2-42.5% (anti-PT ≥8 EU/mL), 85.6-93.8% (anti-FHA ≥8 EU/mL), and 99.5-100% (anti-polio types 1, 2, 3 (≥1:8) [46, 47].

Longer immune persistence data following three primary 3aP-hexa vaccinations and a booster dose in the second year of life have been published [35, 36,48]. Seroprotective antibody levels (among children who had not received additional diphtheria/tetanus booster doses) persisted up to 7-9 years of age in 99.5% of children for Hib (≥0.15 µg/mL), 91.0-97.2% for the three types of poliomyelitis, 66.7% for diphtheria (≥0.1 IU/mL), 72.1-77.2% for HBV (≥10 mIU/mL), and 64.7% for tetanus (≥0.1 IU/mL) [35,36,48]. Seropositivity (≥5 EL.U/mL) for FHA and PRN were high (98.1% and 87.0%, respectively), but for PT, this was only 32.3% [35,36,48]. The low circulating anti-PT antibodies may be indicative of an absence of pertussis infection, suggesting that the vaccination program was effective in preventing pertussis [48]. It is well described that neither natural infection, nor wP or aP vaccines provide life-long protection [25]. Furthermore, studies with both 2aP-hexa and 3aP-hexa indicate waning of pertussis immune response which is consistent with previous reports which reinforce the need for booster vaccination against pertussis [14,18].

Among children in Thailand who had received a birth dose of HBV and three primary doses of 2aP-hexa or 3aP-hexa, 49.3% or 42.9%, respectively, had seroprotective anti-HBs levels at 9-10 years of age [49]. Further, a strong anamnestic response (an enhanced reaction to an antigen related to one previously encountered) was seen post-HBV challenge revaccination in 92.8% and 98.7%, respectively [49]. For 3aP-hexa, immune persistence up to age 14-15 years has been shown after receipt of four doses during infancy (no birth HBV vaccine dose) [50]. Among 268 adolescents, 53.7% and 93.3% had anti-HBs ≥10 mIU/mL before and 1 month after, respectively, a challenge dose of HBV vaccine [50].

Coadministration with Other Childhood Vaccines

If hexavalent combination vaccines are used in the Indian schedule, they would likely be co-administered with rotavirus and/or pneumococcal conjugate vaccines (PCVs) at 6-10-14 weeks [6,7], and could also be co-administered with measles-mumps-rubella (MMR), varicella, measles-mumps-rubella-varicella (MMRV), and/or hepatitis A vaccines in the second year of life [7].

There are no published studies of wP-hexa co-administered with other routine vaccines, but the product leaflet states that it can be given at the same time as PCVs and MMR and rotavirus vaccines [33].

2aP-hexa has been evaluated in co-administration studies outside India, and data suggest no clinically relevant interference on concomitant administration with PCV, MMR, rotavirus, or meningococcal conjugate vaccines [46]. In a South African study in which 15-18-month-old toddlers received a booster dose of 2aP-hexa concomitant with MMR and varicella vaccines, the response to the varicella vaccine was slightly lower than would be expected [32]. Due to a potentially clinically relevant interference in the antibody response of varicella vaccine, 2aP-hexa and varicella vaccines should not be administered at the same time [46].

Various studies have examined the effects on immunogenicity and safety of co-administering 3aP-hexa vaccine with other childhood vaccines outside India: PCVs [51,52] and rotavirus [53], MMRV [54,55], and meningococcal [56-61] vaccines. In all studies, the immune responses remained robust when the vaccines were co-administered, with no clinically relevant interference in the antibody response to each of the antigens [35,36]. Febrile reactions were more common when 3aP-hexa vaccine was administered concomitantly with a PCV or MMRV, but these are mostly moderate (≤39°C) and transient [35,36]. A case-control study reported higher local reactogenicity of 3aP-hexa *vs.* DTaP-IPV-Hib vaccines when co-administered with MMRV vaccine at 18 months of age [55]. For PCV co-administration, fever has been reported to occur less frequently with 3aP-hexa than 2aP-hexa when co-administered with PCV in randomized controlled trials. In one such trial, following a three-dose primary schedule, fever rates of 72.8% (95% CI 67.0-78.1%) and 56.7% (95% confidence interval [CI] 50.5-62.8%) were reported in children who received a PCV plus rotavirus vaccine plus either 2aP-hexa or 3aP-hexa, respectively [62]. A similar trend was seen following the second-year booster vaccination (given with a PCV only), with fever seen in 50.2% (95% CI 43.6-56.8%) and 43.6% (95% CI 37.1-50.3%), respectively [62].

Preterm Infants

3aP-hexa is the only hexavalent vaccine available in India that has prospective clinical data in preterm infants (and includes such information in its label) which indicates that 3aP-hexa has a similar immunogenicity and safety profile in preterm and full-term infants. Cardiorespiratory events in preterm infants of <28 weeks gestation were observed, but this seemed to be influenced by the infantss

underlying condition as the cardiorespiratory risk in this population is a point of attention for the prescriber/ vaccinator in general and most resolved spontaneously or with minimal intervention [63].

CONCLUSIONS

Use of combination vaccines is a practical way to reduce the number of injections given to infants and children. Vaccination schedules can also be simplified with the use of hexavalent combination vaccines for primary and booster vaccination. Three IPV-containing hexavalent vaccines are available in India. The level of available evidence and experience with these three vaccines vary widely [64-66]. All three vaccines evoke immune responses to their contained antigens in phase 3 studies in Indian children using the 6-10-14-week schedule for the primary series [37-39]. 2aP-hexa and 3aP-hexa have also been shown to be immunogenic when tested as a booster dose in the second year of life following a 6-10-14-week primary series [31,32]. All three hexavalent vaccines are well tolerated; although whole-cell pertussis containing vaccines may result in more solicited local reactions and fever than those with acellular pertussis components.

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